

Monitoring Exposure to 4,4'-Methylene-bis(2-chloroaniline) through the Gas Chromatography-Mass Spectrometry Measurement of Adducts to Hemoglobin

by Eric Bailey,¹ Alan G. Brooks,¹ Peter B. Farmer,¹ and Brian Street¹

4,4'-Methylene-bis(2-chloroaniline) (MOCA) is widely used as a curing agent in the plastics industry. The determination of the covalently bound reaction products to hemoglobin (Hb) has been investigated as a biomonitoring method for occupational exposure to this potential human carcinogen. Initial studies using the ¹⁴C-ring-labeled MOCA showed that 24 hr after a single IP dosage to rats (3.74 μ mole/kg), 0.08% of the administered dose was adducted to the Hb, and base hydrolysis liberated 38% of the bound radioactivity. The only product released on hydrolysis was the parent diamine. A specific and sensitive assay procedure using capillary gas chromatography-mass spectrometry has been developed for determining the base-released MOCA adduct down to levels of 20 pmole/g Hb. This method has been used to establish a linear dose-response relationship in IP dosed rats between production of the adduct and dose of MOCA (3.74-44.94 μ mole/kg). It is proposed to use analysis of the Hb adduct as a dosimeter for industrial workers exposed to MOCA.

Introduction

4,4'-Methylene-bis(2-chloroaniline) (MOCA) is a commercially important aromatic amine used in the production of isocyanates, polyurethane foams, and epoxy resins. Of importance with regard to the widespread industrial exposure, which can readily occur through skin absorption, is its reported carcinogenicity in mice, rats, and dogs (1,2). Measurement of MOCA levels in urine (3,4) may be used for biological monitoring, but this only indicates recent exposure, and the results depend critically on the time of sampling. The use of hemoglobin (Hb) adducts as dosimeters for aromatic amines as well as other genotoxic agents allows a retrospective assessment of exposure and gives an indication of the extent of metabolic activation (5-7). The mechanism of adduct formation between aromatic amines and Hb is believed to involve the reaction of *N*-oxidized metabolites of the amine with cysteine residues to form a sulfinic acid amide. Evidence for such reactions *in vitro* has been obtained for MOCA by Chen et al. (8). Sulfinic acid amide adducts can be readily liberated from Hb by mild hydrolysis, yielding the parent amine, the determination of which has been used for assessing human exposure to 4-aminobiphenyl (9), aniline and *p*-chloroaniline (10), and 4,4'-methylenedianiline (MDA) (11).

Measurements of MOCA adducts in rat Hb have been made by radiochemical (12), high-performance liquid chromatography (HPLC) with electrochemical detection (13), gas chromatography-mass spectrometry (GC-MS) (13), and by GC with electron capture detection (8). We report here on an improved GC-MS method, using a stable isotope-labeled internal standard, for the determination of MOCA Hb adducts and report on its application to binding studies in the rat.

Materials and Methods

Ring ¹⁴C-labeled MOCA (specific activity 56 mCi/mmole) was obtained from Amersham International (Amersham, UK). Unlabeled MOCA was recrystallized from aqueous methanol. The purity of both compounds was > 98%. Pentafluoropropionic anhydride (PFPA) (Pierce, Rockford, IL) was used without further purification. All solvents were of Analar grade and were redistilled before use. The synthesis of ³H₆-MOCA used as the internal standard will be described in a forthcoming publication.

Animal Studies

Female LAC Porton-derived Wistar rats (body weight 170-200g, 8-10 weeks old) were administered either ¹⁴C-MOCA or unlabeled MOCA IP in dimethyl sulfoxide. The labeled compound was given at a dose level of 3.74 μ mole/kg and the unlabeled MOCA at doses of 3.74, 11.23, 26.21, and 44.94 μ mole/kg (two animals per dose). Twenty-four hours after dosing, blood sam-

¹MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey SM5 4EF, UK.

Address reprint requests to E. Bailey, MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey SM5 4EF, UK.

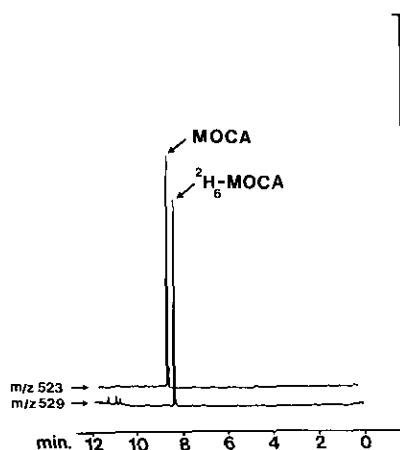


FIGURE 1. Selective ion recording (m/z 523, 529) from the analysis of base-released 4,4'-methylene-bis(2-chloroaniline) (MOCA) in the hemoglobin from a rat given a single IP dose of 3.74 $\mu\text{mole/kg}$ MOCA.

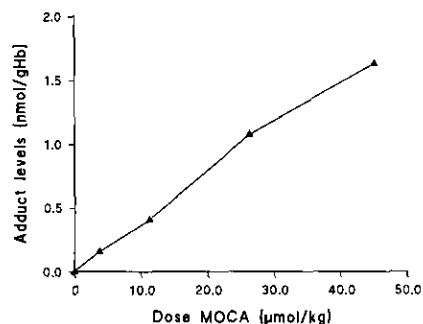


FIGURE 2. Dose-response relationship for the formation of adducted 4,4'-methylene-bis(2-chloroaniline) (MOCA) in hemoglobin after IP exposure of rats to MOCA (3.74–44.94 $\mu\text{mole/kg}$).

ples were collected into heparinized syringes via the descending aorta while animals were under ether anesthesia. Hb was isolated from the blood as described previously (11).

Determination of Hemoglobin Adducts

The analytical method for determining MOCA released by hydrolysis from Hb is based on the GC-MS procedure developed previously for quantifying adducts of MDA to Hb (11). Briefly, it involves the following steps: a) hydrolysis of the Hb sample (20 mg) in 1 N NaOH (4 mL) for 2 hr in the presence of the internal standard $^2\text{H}_6$ -MOCA (5 ng); b) extraction of the hydrolysate three times with ethyl acetate (2.5 mL); c) evaporation of the combined solvent extracts and derivatization with PFPA (3 μL) in ethyl acetate (0.5 mL) for 30 min at room temperature; d) analysis of the derivatized extract by capillary GC (25 m \times 0.32 mm SE 52) with selective ion recording (SIR) MS.

SIR was made in the electron impact mode (EI) monitoring the fragment ions at m/z 523 (M^+-Cl)⁺ derived from the N,N' -disubstituted PFPA derivative of MOCA together with the corresponding fragment ions at m/z 529 from the $^2\text{H}_6$ -MOCA used as the internal standard. Quantitation was made by reference

to calibration curves constructed from the analysis of 20 mg of control rat Hb spiked with $^2\text{H}_6$ -MOCA (5 ng) and from 0 to 30 ng unlabeled MOCA. Peak height ratios m/z 523: m/z 529 were linearly related to the amount of MOCA added (e.g., $r = 0.9995$; $y = 0.1295$; $x = -0.0397$).

Results and Discussion

Twenty-four hours after a single IP dosage of rats with ^{14}C -MOCA (3.74 $\mu\text{mole/kg}$), 0.08% of the administered dose was covalently bound to the Hb. Base hydrolysis liberated 38% of the adducted radioactivity. The only released product observed on thin-layer chromatography with radioscanning was the parent diamine, which was identified by GC-MS after PFPA derivatization. Ethyl acetate extraction of MOCA from Hb hydrolysates gave recoveries of $87.06\% \pm 2.12\%$ (mean \pm SD, $n = 6$). The reaction of MOCA with PFPA was quantitative and the N,N' -di-PFPA derivative had excellent GC properties. Monitoring the intense fragment ion in the EI mass spectrum at m/z 523 (M^+-Cl) allowed 10 pg of the derivatized compound to be detected by SIR. A typical SIR trace from the analysis of the MOCA derived adduct in the Hb of a rat administered a single IP dose of 3.74 $\mu\text{mole/kg}$ MOCA is illustrated in Figure 1. The accuracy of the method was assured by the use of a deuterated analogue of MOCA as an internal standard. The mean calculated recoveries of authentic MOCA spiked into control rat Hb at levels of 7 ng and 25 ng/20 mg Hb were 98.48% (SD \pm 1.33%, $n = 6$) and 100.34% (SD \pm 3.49%, $n = 6$), respectively.

The level of base-released MOCA adduct in rat Hb increased linearly with dose over the range 3.74–44.94 $\mu\text{mole/kg}$ MOCA (Fig. 2). The determined hemoglobin binding index (binding mmole/mole Hb:dose mmole/kg) of the adduct of 2.63 ± 0.204 (mean \pm SEM) was constant over this dose range. In conclusion, the described method should be useful as a dose monitor for industrial workers exposed to MOCA. The detection limit of the method, which is 20 pmole adduct/g Hb may, however, have to be lowered to allow monitoring of low levels of exposure. This can be readily achieved by operating the MS in the negative ion chemical ionization mode, giving a 10-fold improvement in detection limit.

This manuscript was presented as a poster at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October–1 November 1991.

This work was supported by a grant from the UK Health and Safety Executive and from the Commission of the European Communities. We thank D. Gompertz and J. Cocker, The Occupational Medicine Laboratories, Health and Safety Executive (London) for the gift of [^{14}C]MOCA.

REFERENCES

1. Russfield, A. B., Homburger, F., Boger, E., van Dongen, C. G., Weisburger, E. K., and Weisburger, J. H. The carcinogenic effect of 4,4'-methylene-bis(2-chloroaniline) in mice and rats. *Toxicol. Appl. Pharmacol.* 31: 47–54 (1975).
2. Stula, E. F., Barnes, J. R., Sherman, H., Reinhardt, C. F., and Zapp, J. A., Jr. Urinary bladder tumors in dogs from 4,4'-methylene-bis(2-chloroaniline) (MOCA). *J. Environ. Pathol. Toxicol.* 1: 31–50 (1977).
3. Thomas, J. D., and Wilson, H. K. Biological monitoring of workers exposed to 4,4'-methylenebis(2-chloroaniline) (MBOCA). *Br. J. Ind. Med.* 41: 547–551 (1984).
4. Ducos, P., Maire, C., and Gaudin, R. Assessment of occupational exposure to 4,4'-methylene-bis(2-chloroaniline) "MOCA" by a new sensitive method for biological monitoring. *Int. Arch. Occup. Environ. Health* 55: 159–167 (1985).

5. Ehrenberg, L., Moustacchi, E., and Ostermann-Golkar, S. Dosimetry of genotoxic agents and dose-response relationships of their effects. *Mutat. Res.* 123: 121-182 (1983).
6. Farmer, P. B., Neumann, H.-G., and Henschler, D. Estimation of exposure of man to substances reacting covalently with macromolecules. *Arch. Toxicol.* 60: 251-260 (1987).
7. Neumann, H.-G. Biomonitoring of aromatic amines and alkylating agents by measuring hemoglobin adducts. *Int. Arch. Occup. Environ. Health.* 60: 151-155 (1988).
8. Chen, T. H., Kuslikis, B. I., and Braselton, W. E., Jr. Unlabeled hemoglobin adducts of 4,4'-methylenebis(2-chloroaniline) in rats and guinea pigs. *Arch. Toxicol.* 65: 177-185 (1991).
9. Bryant, M. S., Skipper, P. L., Tannenbaum, S. R., and MacIure, M. Hemoglobin adducts of 4-aminobiphenyl in smokers and nonsmokers. *Cancer Res.* 47: 602-608 (1987).
10. Lewalter, J., and Korallus, U. Blood protein conjugates and acetylation of aromatic amines. New findings on biological monitoring. *Int. Arch. Occup. Environ. Health* 56: 179-196 (1985).
11. Bailey, E., Brooks, A. G., Bird, I., Farmer, P. B., and Street, B. Monitoring exposure to 4,4'-methylenedianiline by the gas chromatography-mass spectrometry determination of adducts to hemoglobin. *Anal. Biochem.* 190: 175-181 (1990).
12. Cheever, K. L., Richards, D. E., Weigel, W. W., Begley, K. B., DeBord, D. G., Swearingin, T. F., and Savage, R. E., Jr. 4,4'-Methylene-bis(2-chloroaniline) (MOCA): comparison of macromolecular adduct formation after oral or dermal administration in the rat. *Fundam. Appl. Toxicol.* 14: 273-283 (1990).
13. Sabbioni, G., and Neumann, H.-G. Quantification of haemoglobin binding of 4,4'-methylenebis(2-chloroaniline) (MOCA) in rats. *Arch. Toxicol.* 64: 451-458 (1990).